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Biostimulants Increase Soybean Productivity in the Absence and Presence of Water Deficit in Southern Brazil

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Abstract

Biostimulants offer a potentially novel approach for the regulation/modification of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase yield. The objective of this work was to evaluate the influence of vegetable biostimulants in soybean crop subjected to different soil water conditions. The experiments were carried out in 2017/2018 and 2018/2019, in a completely randomized design (water deficit, combination of biostimulants, and application time). The combinations of biostimulants and time of application were: no combination (control); foliar application at stage V5; foliar application stages V5 and R1; seed treatment; seed treatment and V5 applications; and seed treatment, V5 and R1 applications. All the biostimulant combinations were moreover subject to either the presence or absence of water stress. Evaluations performed were maximum photochemical efficiency, pods per plant, seeds per pod, thousand grain mass, productivity, and incremental increases in performance of each biostimulant treatment. No differences were observed under water deficit in either season, and the use of biostimulants increased the thousand grain mass and final productivity. After two crop seasons with results in increasing yield, the application of biostimulants is recommended in three stages (TS + V5 + R1) for the best management of soybean crops.

Keywords: yield, soil water, plant response, seed treatment

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the world's main agricultural commodity. United States, Brazil and Argentina are the main producers and responsible for 78% of world production of soybean (Faostat, 2016). In 2019/2020 harvest, the estimated production reached 120.4 million tons, an increase of 4.7% relative to 2018/2019. In the southern region of Brazil, there was an increase in the planted area of 1.7% in relation to the previous year; however, climatic problems affected the yield (CONAB, 2020).

Agricultural production is subject to the influence of different climatic adversities, such as low or high temperatures, low light, and excess or lack of rain, the latter being the most limiting factor for soybean production (Fiorenze et al., 2011; Manavalan et al., 2099; Specht et al., 1999). Losses in soybean productivity can be accentuated by water deficiency, which depends on regional climate variability during growth (Schachtman et al., 2008; Das et al., 2017). Water demand during the growing season (120 to 160 days) varies between 450 and 800 mm, with greater demand in the stages between flowering and filling of soybeans; daily demand averages 7.4 mm (Gava et al., 2015). Thus, decreases in soil water availability directly affects soybean yield, in particular during the germination-emergence and flowering-filling stages (Farias et al., 2009).

In this context, several studies have focused on management strategies to reduce the negative effects of soil water deficiency on soybean yield. Among these strategies, the use of plant biostimulants has shown interesting results agroeconomically as well as good acceptance in the market (Jithesh et al., 2012, Cavalcante et al., 2020). These substances are efficient, enhancing the vital processes of the plant and consequently allowing larger harvests and products of better quality. For example, Bertolin et al. (2010), Prieto et al. (2017) and Cavalcante et al. (2020) all observed a positive increase in productivity with the use of biostimulants

Among the biostimulants, extracts of seaweeds such as *Ascophyllum nodosum* (L.) Le Jolis act on plant physiological regulation through different routes, mitigating the effects of environmental stress such as water deficit (Du Jardim, 2015). Extracts of *Ascophyllum nodosum* (L.) Le Jolis consist of cytokinins, auxins, abscisic acid, gibberellins, betaine and alginates (Tarakhovskay et al., 2007; Mackinnon et al., 2010), and can stimulate production of these compounds in plants (Rayorath et al., 2008). A. nodosum has high levels of glycine-betaine (GB) and proline, so it can survive in oceans with a high content of salts in solution. With the use of fermented seaweed extracts in biostimulants, it is intended to provide these osmoprotective compounds for plants. Through these substances, it can interfere in several physiological and/or morphological processes of soybean plants. This interference can occur by the application of these substances via seeds, via soil or via leaves. For this to occur, they need to be absorbed so that they can exert their activity in plants (Klahold et al., 2006; Morais et al., 2020).

The objective of the present study was therefore to evaluate the influence of biostimulants and time of application in soybean crops submitted to different water conditions.

2. Materials and Methods

The experiment was carried out in the 2017/2018 and 2018/2019 growing seasons (November to April) at a Research and Development Experimental area, in Brazil (29º39′59″ S lat, 53º57′41″ W long and altitude of 180 m). According to the Köppen classification, the climate is Cfa and Cfb, humid in all seasons, hot and moderately hot summer, with a predominance of Cfa (Kuinchtner & Buriol, 2001). Throughout the experiment, rainfall data and average temperature were collected daily from a meteorological station installed in the experimental area (Figure 1).

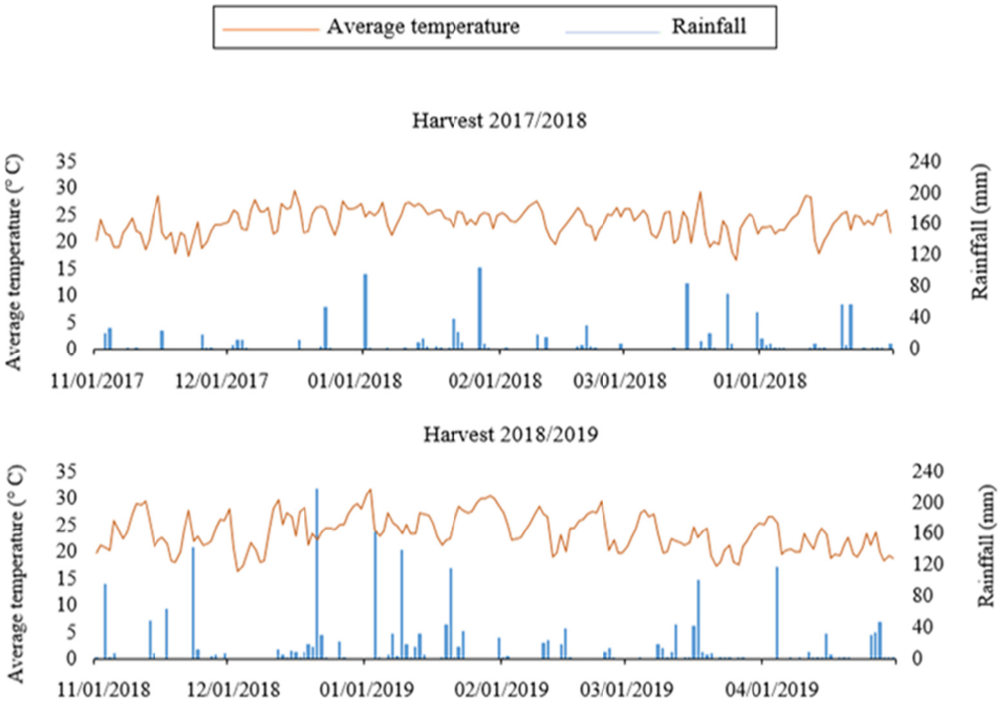

Figure 1. Average temperature and daily rainfall data during the 2017/2018 and 2018/2019 growing seasons in the experimental station

In the experimental area, before soybean sowing, oat (*Avena sativa*) was cultivated in the winter period, where desiccation management was carried out with 4.0 L ha^{-1} of herbicide with the active ingredient glyphosate. The soybean cultivar used was BMX Ponta IPRO (7166 IPRO), with a maturation cycle of 6.6, adapted for the agricultural areas of the central region of the state of Rio Grande do Sul, Brazil. Crop fertilization were based on soil analysis, taking into account recommendations from the Official Soil Analysis Laboratory Network (ROLAS) for soybean, as well as phytosanitary management, which was carried out in accordance with technical recommendations for soybeans in this state.

2.1 Experimental Design

A completely randomized design was adopted, in a 2 × 6 factorial scheme (water deficits x combination of biostimulants and time of application), with four repetitions per treatment. Each experimental plot consisted of five rows of plants spaced 0.5 m apart in an area of 9 m^2 (Figure 2).

Water deficit treatments was either the absence (AD) or presence (PD), and the combinations of biostimulants and time of application were no combination (control); foliar application at the five node stage (V5); foliar application at V5 and initial flowering (R1); seed treatment (TS); TS and V5 applications; TS, V5 and R1 applications (Table 1). The treatments were applied in the phenological stages with greater sanitary demand aiming at productivity.

The vegetable biostimulants used for seed treatment (TS) was Seed$^{+®}$ and for foliar application was Crop$^{+®}$. The five-node stage and initial flowering, designated as stages V5 and R1, were identified according to the scale of Fehr and Caviness (1977). Biostimuland application occurred with a CO_2 sprayer with a flow rate of 150 L ha^{-1}. The biostimulants were obtained from concentrates of the seaweed *Ascophyllum nodosum* (L.) Le Jolis (Misra et al., 2017).

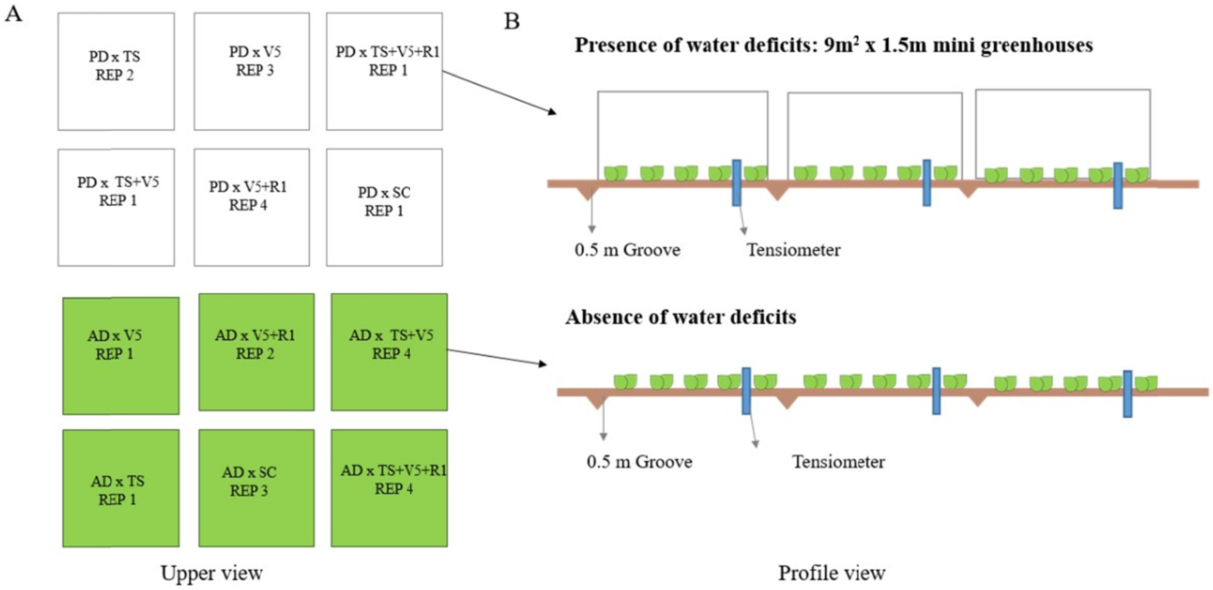

Figure 2. Sketch of the experimental area with the distribution of treatments (water deficits and combinations of uses of biostimulants and timing of application), in the 2017/2018 and 2018/2019 harvests, in the municipality of Santa Maria, RS

Table 1. Combinations of biostimulant uses and timing of application for both water deficit conditions

Combinations of biostimulant uses and timing of application	Bioestimulant
SC: no combination use (witness)	-
V5: only application via foliar part in the phenological stage of the fifth node	^{B}Crop$^{+®}$
V5+R1: application via foliar part in the phenological stages of the fifth node and beginning of flowering	Crop$^{+®}$ + Crop$^{+®}$
TS: only seed treatment	^{A}Seed$^{+®}$
TS+V5: seed treatment and foliar application in the phenological stage of the fifth node	Seed$^{+®}$ + Crop$^{+®}$
TS+V5+R1: seed treatment, foliar application in the phenological stages of the fifth node and beginning of flowering	Seed$^{+®}$ + Crop$^{+®}$ + Crop$^{+®}$

Note. ^{A} Seed+® Dose: 2.0 ml kg^{-1} de sementes. ^{B} Crop$^{+®}$ Dose: 250.0 ml ha^{-1}.

To compose the treatments, two plant biostimulants were used—one applied via seed treatment in the soybean crop (Seed+®) and another with application via foliar (Crop+®), in stages V5 and R1 of the crop.

2.2 Soil Water Deficit Management

Soil water deficits were induced between stages R2 (full bloom) and R6 (green soybean or full pod), according to the phenological scale proposed by Fehr and Caviness (1977). For the management of the soil water deficit, mini-greenhouses were built of polypropylene plastic with 1.5 m height (Figure 2). Ditches of 0.5 m deep were made surrounding the experimental plots in order to reduce the possibility of water entering through lateral flow.

Soil moisture was monitored by HidroFarm sensors and soil water tension by tensiometers. The Halk Farm sensors (Falker brand) measure volumetric soil water content by ISAF technology and directly report the value of the volumetric moisture of the soil in percentage (Faraco et al., 2016). For experimental areas that were not subjected to water deficiency, water was replaced as soon as volumetric moisture reached 17.6% (1% above the critical humidity for the type of soil and the crop used (16.6%)). Through monitoring with tensiometers in the experimental area, irrigation was carried out when the water tension exceeded the 70 kPa range (water deficiency).

Based on the following soil physical analysis, irrigation parameters were chosen: Texture (Sand 68.9%, Silt 21.1% and Clay 10%); WHC (%) 21.3; PWP (%) 9.5 (Santos et al., 2015; Bergamaschi et al., 1992). Experimental plots were irrigated by a drip irrigation system in each sowing line of the soybean crop. Experimental plots under water deficiency were maintained with a volumetric moisture content below 16.6% and water tension between 20 and 30 kPa.

2.3 Biometric Parameters

The maximum photochemical efficiency of PSII (Fv/Fm) was calculated using the variable fluorescence ratio (Fm-Fo) and the maximum fluorescence. The Fv/Fo ratio was calculated using the variable fluorescence ratio (Fm-Fo) and initial fluorescence.

Using the parameters of initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence/initial fluorescence ratios (Fv/Fo) were measured with a modulated pulse fluorometer (Junior-PAM, Walz, Effeltrich, Germany), in the period between 3 hand 8 h. The leaves were pre-adapted in the dark for 30 minutes for initial fluorescence readings (Fo) and maximum fluorescence (Fm) was measured by means of a pulse of saturating light (10,000 μmol m^{-2} s^{-1}) for 0.6 seconds.

Production components were evaluated at full maturity, including the number of pods $plant^{-1}$ and the number of soybeans pod^{-1}, through the random collection of 10 plants in the useful area ofeach experimental plot. At the R9 stage (harvest maturity), the plants were harvested manually and the mass of a thousand soybeans and the soybean yield were evaluated in the experimental units where the productivity in kg ha^{-1} was calculated.

The increment index for pods $plant^{-1}$ (II.BIO), adapted and determined by the methodology of Menegaes et al. (2019), is expressed in Equation 1:

$$\text{II.PODS} = ((\text{PODS}_{CC} - \text{PODS}_{SC})/\text{PODS}_{SC}) \times 100 \tag{1}$$

Where, PODS_{CC} refers to each combination of biostimulants and time of application, and PODS_{SC}: refers to the control treatment. The productivity increment index (II.PROD), adapted and determined by the methodology of Menegaes et al. (2019), is expressed in Equation 2:

$$\text{II.PROD} = ((\text{PROD}_{CC} - \text{PROD}_{SC})/\text{PROD}_{SC}) \times 100 \tag{2}$$

Where, PROD_{CC}: soybean production of each combination of biostimulants and time of application, and PROD SC: soybean production in control treatment.

For both the 2017/2018 and 2018/2019 harvests, the data obtained were subjected to analysis of variance (ANOVA) individually, according to the mathematical model of the randomized block design with factorial arrangement, with the unfolding of the variables that presented a significant response to the interaction between the factors studied, as well as a comparison of the means by Tukey test ($p < 0.05$), using the SISVAR program (Ferreira, 2014).

3 Results and Discussion

3.1 Photosynthetic Responses

The average potential quantum efficiency (Fv/Fm) of soybean plants was 0.83 and 0.79 in the absence and presence of the water deficit, respectively (Table 2), during 2018/2019. The use of biostimulants spread across two to three applications (V5 + R1, TS + V5 and TS + V5 + R1) showed a greater potential quantum efficiency ratio for both water deficit conditions.

Table 2. Average potential quantum efficiency (Fv/Fm) of soybean plants subjected to different water deficits and combinations of biostimulants and application times in the 2018/2019 harvest, and summary of the analysis of variance

Combinations	Water deficit		
	Absence	Presence	Average
SC	0.81 Ac*	0.77 Bb	0.79
V5	0.82 Abc	0.79 Ba	0.81
V5 + R1	0.83 Aab	0.80 Ba	0.82
TS	0.82 Abc	0.79 Ba	0.81
TS + V5	0.85 Aa	0.80 Ba	0.82
TS + V5 + R1	0.84 Aa	0.81 Ba	0.83
Average	0.83	0.79	
	DF	MSE	p-value
Water deficit (W)	1	0.014352	0.0000 *
Combinations (C)	5	0.001494	0.0000 *
Interaction W*C	5	0.000187	0.0500 *
Residual	36	0.000076	
CV (%)			

Note. * Means followed by the same uppercase letter (in the same row) or by the same lowercase letter (in the same column) are not significantly different. DF: degrees of freedom; MSE: mean squared error; CV (%): coefficient of variation. SC: control with no biostimulants; TS: seed treatment only; TS+V5: seed treatment and foliar application at V5; TS + V5 + R1: seed treatment and foliar application at V5 and R1; V5: foliar application only at V5; V5 + R1: foliar application at V5 and R1.

Mehta et al. (2001) point out that the increases in the values of the Fv/Fm ratio indicate an increase in the efficiency of photosynthetic conversion of the PSII. For Shu et al. (2013), in physiologically balanced plants, that is, plants in stress-free conditions, the values of Fv/Fm are approximately 0.75, whereas Kalaji (2008) suggests that values lower than this may indicate that the plants were exposed to some type of biotic or abiotic stress that reduced photochemical capacity.

In general, the reduction in available soil water influenced the photochemical efficiency of photosystem II, as evidenced by the Fv/Fm ratio, so that plants under water stress suffered damage to their photosynthetic apparatus. This was also observed by Molinari et al. (2007) with sugar cane plants (*Saccharum officinarum* L.) subjected to water stress, which showed decreases in the quantum yield of photosystem photochemistry. Caires et al. (2010) stated that water deficiency can affect chlorophyll levels and their fluorescence, corroborating the results found in this work.

3.2 Productivity

In both the 2017/2018 and 2018/2019 harvests, neither the number of pods plant^{-1} nor the number of soybeans pod^{-1} showed statistically significant differences (^{ns}) (Table 3).

Table 3. Number of pods plant^{-1} and number of soybeans pod $^{-1}$ of soybean crops subjected to different water deficits and combinations of biostimulants and times of application in the 2017/2018 and 2018/2019 harvests, and summary of the analysis of variance

Combinations	Water deficit					
	Harvest 2017/2018			Harvest 2018/2019		
	Absence	Presence	Average	Absence	Presence	Average
Pods plant^{-1}						
SC	46.0 ^{ns}	43.3	44.6 c	75.0 ^{ns}	53.5	64.3b
V5	70.8	66.3	68.5 ab	71.8	47.8	59.8 b
V5 + R1	70.8	67.8	69.3 ab	79.8	52.5	66.1 b
TS	61.3	55.0	58.1 bc	101.5	50.5	76.0 b
TS + V5	75.3	70.0	72.6 ab	91.5	93.0	92.3 b
TS + V5 + R1	79.5	74.0	76.8 a	176.3	110.5	143.4 a
Average	67.25 A	62.71 A		99.29 A	67.96 B	
		DF	MSE	p-value	DF	MSE
Water deficit (W)	1	247.520833	0.1837 ^{ns}	1	11781.333333	0.0014 *
Combinations (C)	5	1.102270833	0.0000 *	5	7926.800000	0.0000 *
Interaction W*C	5	3.970833	0.9996 ^{ns}	5	1126.583333	0.3543 ^{ns}
Residuals	36	134.729167		36	982.861111	
CV (%)	17.86			37.49		
Soybeans pod^{-1}						
SC	2.2^{ns}	2.0	2.1 c	2.4 ^{ns}	2.4	2.4 a
V5	2.4	2.3	2.3 bc	2.6	2.4	2.5 a
V5 + R1	2.4	2.4	2.4 ab	2.5	2.3	2.4 a
TS	2.3	2.2	2.3 bc	2.6	2.5	2.5 a
TS + V5	2.6	2.4	2.5 a	2.6	2.1	2.4 a
TS + V5 + R1	2.6	2.5	2.5 a	2.5	2.4	2.5 a
Average	2.42 A	2.30 B		2.52 A	2.36 B	
	DF	MSE	p-value	DF	MSE	p-value
Water deficit (W)	1	0.175208	0.0213 *	1	0.312019	0.0004 *
Combinations (C)	5	0.206625	0.0001 *	5	0.034342	0.1586 ^{ns}
Interaction W*C	5	0.012208	0.8430 ^{ns}	5	0.048719	0.0546 ^{ns}
Residuals	36	30.243		36	0.020145	
CV (%)	7.38			5.82		

Note. * Significant interaction and ^{ns} nonsignificant interaction of factors. Means followed by the same uppercase letter (in the same row) or by the same lowercase letter (in the same column) are not significantly different. DF: degrees of freedom; MSE: mean squared error; CV (%): coefficient of variation. SC: control with no biostimulants; TS: seed treatment only; TS+V5: seed treatment and foliar application at V5; TS + V5 + R1: seed treatment and foliar application at V5 and R1; V5: foliar application only at V5; V5 + R1: foliar application at V5 and R1.

Averages of the number of pods plant^{-1} were 67.2 and 62.7 for the absence and presence of water deficit, respectively, in 2017/2018, and 99.2 and 67.9 for absence and presence of the water deficit, respectively, in 2018/2019. In the 2018/2019 harvest, the average number of pods plant^{-1} in the absence of water deficit stood out from the others, especially when using biostimulants at three different times of application (TS + V5 + R1), reaching a value of 176.3 pods plant^{-1}. These results corroborate with those found by Klahold et al. (2006), who observed an increase in the number of pods plant^{-1} relative to the control treatment with the application of biostimulants via seed and foliar application.

The results obtained in both harvests show that the use of biostimulants at different application times helped the plants form and fill out the pods, for a positive effect on productivity.

The average numbers of soybean soybeans pod^{-1} were 2.42 and 2.30 in the absence and presence of water deficit, respectively, in the 2017/2018 harvest, and 2.52 and 2.36 in the 2018/2019 harvest (Table 2). In both harvests, water deficit induced a lower number of soybeans pod $^{-1}$ in relation to the absence of water deficit for all combinations of biostimulants and application times.

Cavalcante et al. (2020) also observed similarity in the number of soybeans pod^{-1} in soybean plants treated with different biostimulants and water deficits. The authors concluded that the simple use of biostimulants led to improvements in the physiological protection of plants under water stress.

There was no statistical significance among values for the weight of one thousand soybeans (Table 4), however, for both 2017/2018 and 2018/2019 harvests, the general averages regarding absence and presence of water deficit were similar. The mass of a thousand soybeans is directly related to the final productivity of the crop, indicating the success of cultural management during the cultivation phase, in which the crop is subjected to different environmental conditions, including water availability.

The use of biostimulants spread over two to three applications (TS + V5 and TS + V5 + R1) led to greater value for the mass of one thousand soybeans for both water deficit conditions. This may be related to the effect of using biostimulants, which provided better conditions for the production of photoassimilates, with mitigation of environmental stress from protection via seeds. Thus, these data corroborate the work of Bertolin et al. (2010) and Prieto et al. (2017), who also used biostimulants as a treatment for soybean seeds.

Average productivity was 3,579 and 3,411 kg ha^{-1} in the absence and presence of water deficit for the 2017/2018 crop, and 4,376 and 3,915 kg ha^{-1} in the 2018/2019 harvest (Table 5). It was found that in the 2017/2018 crop, the use of biostimulants spread across three applications (TS + V5 + R1) in the absence of water deficit demonstrated an average productivity of 3,907 kg ha^{-1} in relation to the other treatments. In the 2018/2019 harvest, there was a significant difference among treatments, with notable increases in productivity from two to three applications of biostimulants (TS + V5 and TS + V5 + R1), averaging with averages of 4.437 and 4.462 kg ha^{-1}, respectively.

Our data corroborate the work of Dourado Neto et al. (2004) and Lana et al. (2009), who observed that the use of biostimulants favored the productivity of corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.) respectively, when carried out as seed or foliar treatment.

Table 5 shows the incremental increases in production parameters for soybean crops, for both harvests, in relation to the control treatment. The water conditions of the crops were similar in both harvests. There was a noteable increase of 74.5% and 127.0% in the number of pods plant^{-1} for the combination of biostimulants with three flowering applications (TS + V5 + R1) in relation to the SC treatment, for the 2017/2018 and 2018/2019 crops, respectively.

Use of biostimulant as seed treatment (TS) in a single application, in both harvests, presented the smallest increase in productivity in relation to the control treatment. However, the other combinations of biostimulants and times of application all showed a positive effect on soybean yield.

Differences in results between the two harvests (2017/2018 and 2018/2019) reflect the different climatic conditions. It can be suggested that ecophysiological factors, both together and in isolation, affect the performance of biostimulants, either through seed treatment and/or by foliar application. Klahold et al. (2006) found that the application of a bioregulator via seed treatment and/or foliar route led to an increase in the number of pods, the number of soybeans and the overall production per plant.

Thus, there is a clear benefit to the application of biostimulants in soybean, in particular in maintaining productivity when plants are subjected to climatic adversities, such as water deficiency, at critical stages for the crop. Our results corroborate those of Bertolin et al. (2010), who observed that the application of biostimulants provided an increase in soybean productivity via seed and foliar application, in addition to the increase in the number of pods plant^{-1}.

Table 4. Mass per thousand grain and soybean yield under different water deficits and combinations of biostimulants and application times in the 2017/2018 and 2018/2019 seasons, and summary of the analysis of variance

Combinations	Water deficit					
	Harvest 2017/2018			Harvest 2018/2019		
	Absence	Presence	Average	Absence	Presence	Average
Mass of a thousand soybeans (g)						
SC	154.2 ^{ns}	151.5	152.9 c	153.9 ^{ns}	151.3	152.6 e
V5	162.6	162.6	162.6 b	169.6	168.6	169.1 c
V5 + R1	167.8	166.4	167.1 b	173.5	172.8	173.2 b
TS	158.2	153.2	155.7 c	162.5	159.3	160.9 d
TS + V5	175.7	174.6	175.2 a	173.1	171.7	172.4 ab
TS + V5 + R1	178.4	178.6	178.5 a	179.0	177.6	178.3 a
Média	166.14 A	164.47 A		168.60 A	166.89 B	
		DF	MSE	p-value	DF	MSE
Water deficit (W)	1	33.600533	0.0743 ^{ns}	1	34.901352	0.0327 *
Combinations (C)	5	847.384748	0.0000 *	5	702.739302	0.0000 *
Interaction W*C	5	7.689283	0.5753 ^{ns}	5	1.879142	0.9289 ^{ns}
Residue	36	9.943014		36	7.075566	
CV (%)	1.16			1.59		
Productivity (kg ha^{-1})						
SC	3.297 ^{ns}	3.143.8	3,220.6 c	3,909.6 Ab*	3,270.4 Bb	3,590.0
V5	3.609	3.504.3	3,557.0 ab	4,393.1 Aab	4,117.4 B	4,255.2
V5 + R1	3.603	3.476.8	3,540.3 ab	4,399.5 Aab	4,254.7 Aaa	4,327.1
TS	3.360.1	3.300.2	3,330.1 bc	4,270.0 Aab	3,342.5 Bb	3,806.3
TS + V5	3.700.8	3.461.7	3,581.3 b	4,618.6 Aa	4,255.5 Ba	4,437.0
TS + V5 + R1	3.907.6	3.579.5	3,743.5 a	4,669.8 Aa	4,255.5 Ba	4,462.6
Média	3.579.92 A	3.411.04 B		4,376.76	3,915.99	
		DF	MSE	p-value	DF	MSE
Water deficit (W)	1	342253.896852	0.0012 *	1	2547740.168802	0.0000 *
Combinations (C)	5	284136.774494	0.0000 *	5	1046803.258277	0.0000 *
Interaction W*C	5	19270.803187	0.6294 ^{ns}	5	158173.287127	0.0454 *
Residue	36	27661.469349		36	62245.549908	
CV (%)	4.76					

Note. * Significant interaction and ^{ns} nonsignificant interaction of factors. Means followed by the same uppercase letter (in the same row) or by the same lowercase letter (in the same column) are not significantly different. DF: degrees of freedom; MSE = mean squared error; CV (%): coefficient of variation. SC: control with no biostimulants; TS: seed treatment only; TS+V5: seed treatment and foliar application at V5; TS + V5 + R1: seed treatment and foliar application at V5 and R1; V5: foliar application only at V5; V5 + R1: foliar application at V5 and R1.

Table 5. Percentage increase in the number of pods plant^{-1} and productivity of soybeans under different water deficits and combinations of biostimulants and times of application, and summary of the analysis of variance

Combinations	Water deficit					
	Harvest 2017/2018			Harvest 2018/2019		
	Absence	Presence	Average	Absence	Presence	Average
Percent increase in pods plant^{-1} in relation to control						
V5	53.8 ^{ns}	57.7	55.8 ab	3.8 ^{ns}	7.2	5.5 b
V5 + R1	53.8	61.3	57.6 ab	11.3	7.7	9.5 b
TS	33.2	31.0	32.1 b	39.0	5.8	22.4 b
TS + V5	63.6	66.7	65.1 ab	25.3	78.8	52.1 b
TS + V5 + R1	72.8	76.2	74.5 a	141.4	112.5	127.0 a
Average	55.4 A	58.6 A		44.2 A	42.4 A	
	DF	MSE	p-value	DF	MSE	p-value
Water deficit (W)	1	98.470440	0.7022 ^{ns}	1	31.488503	0.9064 ^{ns}
Combinations (C)	4	1993.468785	0.0333 *	4	20172.712188	0.0001 *
Interaction W*C	4	24.133328	0.9973 ^{ns}	4	2408.261877	0.3864 ^{ns}
Residue	30	660.934292		30	2240.699383	
CV (%)	11.27			27.36		
Percent increase in productivity in relation to control						
V5	11.5 ^{ns}	12.3	11.9 ab	13.6 Ba*	27.8 Aa	20.7
V5 + R1	11.3	11.4	11.3 ab	13.7 Ba	32.1 Aa	22.9
TS	3.8	5.8	4.8 b	10.4 A a	4.4 B b	7.4
TS + V5	14.3	10.9	12.6 ab	19.4 Ba	32.1 Aa	25.7
TS + V5 + R1	20.7	14.7	17.7 a	20.7 Ba	32.1 Aa	26.4
Média	12.3 A	11.0 A		15.6	25.7	20.6
	DF	MSE	p-value	DF	MSE	p-value
Water deficit (W)	1	16.525103	0.4760 ^{ns}	1	1024.548840	0.0002 *
Combinations (C)	4	169.799004	0.0022 *	4	479.313254	0.0001 *
Interaction W*C	4	21.748334	0.6075 ^{ns}	4	175.813034	0.0272 *
Residue	30	31.721458		30	55.267602	
CV (%)	12.08			9.01		

Note. * Significant interaction and ^{ns} nonsignificant interaction of factors. Means followed by the same uppercase letter (in the same row) or by the same lowercase letter (in the same column) are not significantly different. DF: degrees of freedom; MSE:mean squared error; CV (%): coefficient of variation. SC: control with no biostimulants; TS: seed treatment only; TS+V5: seed treatment and foliar application at V5; TS + V5 + R1: seed treatment and foliar application at V5 and R1; V5: foliar application only at V5; V5 + R1: foliar application at V5 and R1.

4. Conclusions

The use of biostimulants at different times of application in soybeans crop improves potential photosynthetic quantum efficiency and the resulting physiological response.

Application of biostimulants as part of soybean management is recommended at three times, namely in the treatment of seeds and then as foliar application at the V5 and R1 stages.

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